

FIGURE 1

cDNA sequence of wild type amFP486

ATGGCTCTTTCAAACAAGTTTATCGGAGATGACATGAAAATGACCTACCATATGGATG
GCTGTGTCAATGGGCATTACTTTACCGTCAAAGGTGAAGGCAACGGGAAGCCATACGA
AGGGACGCAGACCTCGACTTTTAAAGTCACCATGGCCAACGGTGGGCCCCCTTGCAATC
TCCTTTGACATACTATCTACAGTGTTCAAGTATGGAAATCGATGCTTTACTGCGTATC
CTACCAGTATGCCCCGACTATTTCAAACAAGCATTTTCCTGACGGAATGTCATATGAAAG
GACTTTTACCTATGAAGATGGAGGAGTTGCTACAGCCAGTTGGGAAATAAGCCTTAAA
GGCAACTGCTTTGAGCACAAATCCACGTTTCATGGAGTGAACTTTCCTGCTGATGGAC
CTGTGATGGCGAAGATGACAACCTGGTTGGGACCCATCTTTTGAGAAAATGACTGTCTG
CGATGGAATATTGAAGGGTGATGTCACCGCGTTCCTCATGCTGCAAGGAGGTGGCAAT
TACAGATGCCAATTCACACTTCTTACAAGACAAAAAACCGGTGACGATGCCACCAA
ACCATGCGGTGGAACATCGCATTGCGAGGACCGACCTTGACAAAGGTGGCAACAGTGT
TCAGCTGACGGAGCACGCTGTTGCACATATAACCTCTGTTGTCCCTTTC (SEQ ID
NO: 01)

amino acid sequence of wild type amFP486

MALSNKFIGD DMKMTYHMDG CVNGHYFTVK GEGNGKPYEG TQTSTFKVTM ANGGPLAFSF
DILSTVFKYG NRCFTAYPTS MPDYFKQAFP DGMSYERTFT YEDGGVATAS WEISLKGNCF
EHKSTFHGVN FPADGPVMAK MTTGWDPSFE KMTVCDGILK GDVTAFLMLQ GGGNYRCQFH
TSYKTKKPVT MPPNHAVEHR IARTDLKGG NSVQLTEHAV AHITSVVPF
(SEQ ID NO: 02)

Figure 2

cDNA sequence of zFP506

ATGGCTCAGTCAAAGCACGGTCTAACAAAAGAAATGACAATGAAATACCGTATGGAAGGGTGC
GTCGATGGACATAAATTTGTGATCACGGGAGAGGGCATTGGATATCCGTTCAAAGGGAAACAG
GCTATTAATCTGTGTGTGGTCTGAAGGTGGACCATTTGCCATTTGCCGAAGACATATTGTCAGCT
GCCTTTTATGTACGGAAACAGGGTTTTCTACTGAATATCCTCAAGACATAGCTGACTATTTCAAG
AACTCGTGTCCTGCTGGTTATACATGGGACAGGTCTTTTCTCTTTGAGGATGGAGCAGTTTGC
ATATGTAATGCAGATATAACAGTGAGTGTTGAAGAAAAGTGCATGTATCATGAGTCCAAATTT
TATGGAGTGAATTTTCTCTGCTGATGGACCTGTGATGAAAAAGATGACAGATAACTGGGAGCCA
TCCTGCGAGAAGATCATACCAGTACCTAAGCAGGGGATATTGAAAGGGGATGTCTCCATGTAC
CTCCTTCTGAAGGATGGTGGGCGTTTACGGTGCCAATTCGACACAGTTTACAAAGCAAAGTCT
GTGCCAAGAAAGATGCCGGACTGGCACTTCATCCAGCATAAGCTCACCCGTGAAGACCGCAGC
GATGCTAAGAATCAGAAATGGCATCTGACAGAACATGCTATTGCATCCGGATCTGCATTGCCC
(SEQ ID NO:03)

amino acid sequence of zFP506

MAQSKHGLTK EMTMKYRMEG CVDGHKFVIT GEGIGYPFKG KQAINLCVVE GGPLPFAEDI LSAAFNYGNR VFTEYPQDIA
DYFKNSCPAG YTWDRSFLFE DGAVCICNAD ITVSVEENCM YHESKFYGVN FPADGPVMKK MTDNWEPSCE KIIPVPKQGI
LKGDVSMYLL LKDGGRRLRCQ FDTVYKAKSV PRKMPDWHFI QHKLTREDRS DAKNQKWHLT EHAIASGSAL P
(SEQ ID NO:04)

Figure 3

cDNA sequence of zFP538

gagttgagtt tctcgacttc agttgtatca attttggggc atcaagcgat ctattttcaa
catggctcat tcaaagcacg gtctaaaaga agaaatgaca atgaaatacc acatggaagg
gtgcgtcaac ggacataaat ttgtgatcac gggcgaaggc attggatatc cgttcaaagg
gaaacagact attaatctgt gtgtgatcga agggggacca ttgccatttt ccgaagacat
attgtcagct ggctttaagt acggagacag gatttttact gaatattctc aagacatagt
agactatttc aagaactcgt gtctgtctgg atatacatgg ggcaggctct ttctctttga
ggatggagca gtctgcatat gcaatgtaga tataacagtg agtgtcaaag aaaactgcat
ttatcataag agcatattta atggaatgaa ttttcctgct gatggacctg tgatgaaaaa
gatgacaact aactgggaag catcctgcga gaagatcatg ccagtaccta agcaggggat
actgaaaggg gatgtctcca tgtacctcct tctgaaggat ggtgggcgtt accggtgcca
gttcgacaca gtttacaag caaagtctgt gccaaagtaag atgccggagt ggcacttcat
ccagcataag ctctccgtg aagaccgcag cgatgctaag aatcagaagt ggcagctgac
agagcatgct attgcattcc cttctgcctt ggcttgataa gaatgtagtt ccaacatttt
aatgcatgtg cttgtcaatt attctgataa aaatgtagtt gagttgaaaa cagacaagta
caaataaagc acatgtaa at cgtct (SEQ ID NO:05)

amino acid sequence of zFP538

Met Ala His Ser Lys His Gly Leu Lys Glu Glu Met Thr Met Lys
Tyr His Met Glu Gly Cys Val Asn Gly His Lys Phe Val Ile Thr
Gly Glu Gly Ile Gly Tyr Pro Phe Lys Gly Lys Gln Thr Ile Asn
Leu Cys Val Ile Glu Gly Gly Pro Leu Pro Phe Ser Glu Asp Ile
Leu Ser Ala Gly Phe Lys Tyr Gly Asp Arg Ile Phe Thr Glu Tyr
Pro Gln Asp Ile Val Asp Tyr Phe Lys Asn Ser Cys Pro Ala Gly
Tyr Thr Trp Gly Ser Phe Leu Phe Glu Asp Gly Ala Val Cys Ile
Cys Asn Val Asp Ile Thr Val Ser Val Lys Glu Asn Cys Ile Tyr
His Lys Ser Ile Phe Asn Gly Met Asn Phe Pro Ala Asp Gly Pro
Val Met Lys Lys Met Thr Thr Asn Trp Glu Ala Ser Cys Glu Lys
Ile Met Pro Val Pro Lys Gln Gly Ile Leu Lys Gly Asp Val Ser
Met Tyr Leu Leu Leu Lys Asp Gly Gly Arg Tyr Arg Cys Gln Phe
Asp Thr Val Tyr Lys Ala Lys Ser Val Pro Ser Lys Met Pro Glu
Trp His Phe Ile Gln His Lys Leu Leu Arg Glu Asp Arg Ser Asp
Ala Lys Asn Gln Lys Trp Gln Leu Thr Glu His Ala Ile Ala Phe
Pro Ser Ala Leu Ala (SEQ ID NO:06)

FIGURE 5

Amino Acid and Nucleotide Sequence for asFP600

ATGGCTTCCTTTTTTAAAGAAGACTATGCCCTTTAAGACGACCATTGAAGGGACGGTTAATGGCCAC
TACTTCAAGTGTACAGGAAAAGGAGAGGGCAACCCATTTGAGGGTACGCAGGAAATGAAGATAGAG
GTCATCGAAGGAGGTCCATTGCCATTTGCCTTCCACATTTTGTCAACGAGTTGTATGTACGGTAGT
AAGGCCTTCATCAAGTATGTGTGTCAGGAATTCTTGACTACTTCAAGCAGTCTTTCCTGAAGGTTTT
ACTTGGGAAAGAACCACAACCTACGAGGATGGAGGCTTCTTACAGCTCATCAGGACACAAGCCTA
GATGGAGATTGCCTCGTTTACAAGGTCAAGATTCTTGGTAATAATTTTCCTGCTGATGGCCCCGTG
ATGCAGAACAAAGCAGGAAGATGGGAGCCATCCACCGAGATAGTTTATGAAGTTGACGGTGTCTTG
CGTGGACAGTCTTTGATGGCCCTTAAGTGCCCTGGTGGTCGTCATCTGACTTGCCATCTCCATACT
ACTTACAGGTCCAAAAAACCAGCTGCTGCCTTGAAGATGCCAGGATTTTCAATTTTGAAGATCATCGC
ATCGAGATAATGGAGGAAGTTGAGAAAGGCAAGTGCTATAAACAGTACGAAGCAGCAGTGGGCAGG
TACTGTGATGCTGCTCCATCCAAGCTTGGACATAAC (SEQ ID NO:09)

Amino acid

MASFLKKTMP FKTITIEGTVN GHYFKCTGKG EGNPFEGTQE MKIEVIEGGP LPFAFHILST
SCMYGSKTFI KYVSGIPDYF KQSFPEGFTW ERTTTYEDGG FLTAHQDTSI DGDCLVYKVK
ILGNNFPADG PVMQNKAGRW EPATEIVYEV DGVLRGQSLM ALKCPGGRHL TCHLHTTYRS
KKPAAALKMP GFHFEDHRIE IMEEVEKGKC YKQYEA AVGR YCDAAPSKLG HN (SEQ ID
NO:10)

Figure 6

Sequence of humanized 6/9 hybrid gene and 6/9-Q3 mutant

	for 6/9-2G and 6/9-Q3 CAG (Q)																
1	ATG	AGC	TGC	AGC	AAG	AAC	GTG	ATC	AAG	GAG	TTC	ATG	CGG	TTC	AAG	GTG	48
1	M	S	C	S	K	N	V	I	K	E	F	M	R	F	<u>K</u>	V	16
49	CGG	ATG	GAG	GGC	ACC	GTG	AAC	GGC	CAC	GAG	TTC	GAG	ATC	AAG	GGC	GAG	96
17	R	M	E	G	T	V	N	G	H	E	F	E	I	K	G	E	32
97	GGC	GAG	GGC	CGG	CCC	TAC	GAG	GGC	CAC	TGC	AGC	GTG	AAG	CTC	ATG	GTG	144
33	G	E	G	R	P	Y	E	G	H	C	S	V	K	L	M	V	48
145	ACC	AAG	GGC	GGC	CCC	CTC	CCC	TTC	GCC	TTC	GAC	ATC	CTC	AGC	CCC	CAG	192
49	T	K	G	G	P	L	P	F	A	F	D	I	L	S	P	Q	64
193	TTC	CAG	TAC	GGC	AGC	AAG	GTG	TAC	GTG	AAG	CAC	CCC	GCC	GAC	ATC	CCC	240
65	F	Q	Y	G	S	K	V	Y	V	K	H	P	A	D	I	P	80
ATG (M) for 6/9-Q3																	
241	GAC	TAC	AAG	AAG	CTC	AGC	TTC	CCC	GAG	GGC	TTC	AAG	TGG	GAG	CGG	GTG	288
81	D	Y	<u>K</u>	K	L	S	F	P	E	G	F	K	W	E	R	V	96
289	ATG	AAC	TTC	GAG	GAC	GGC	GGC	GTG	GTG	ACC	GTG	AGC	CAG	GAC	AGC	AGC	336
97	M	N	F	E	D	G	G	V	V	T	V	S	Q	D	S	S	112
337	CTC	AAG	GAC	GGC	TGC	TTC	ATC	TAC	GAG	GTG	AAG	TTC	ATC	GGC	GTG	AAC	384
113	L	K	D	G	C	F	I	Y	E	V	K	F	I	G	V	N	128
385	TTC	CCC	AGC	GAC	GGC	CCC	GTG	ATG	CAG	CGG	CGG	ACC	CGG	GGC	TGG	GAG	432
129	F	P	S	D	G	P	V	M	Q	R	R	T	R	G	W	E	144
433	GCC	AGC	AGC	GAG	CGG	CTC	TAC	CCC	CGG	GAC	GGC	GTG	CTC	AAG	GGC	GAC	480
145	A	S	S	E	R	L	Y	P	R	D	G	V	L	K	G	D	160
481	ATC	CAC	ATG	GCC	CTC	CGG	CTC	GAG	GGC	GGC	GGC	CAC	TAC	CTC	GTG	GAG	528
161	I	H	M	A	L	R	L	E	G	G	G	H	Y	L	V	E	176
529	TTC	AAG	AGC	ATC	TAC	ATG	GCC	AAG	AAG	CCC	GTG	CAG	CTC	CCC	GGC	TAC	576
177	F	K	S	I	Y	M	A	K	K	P	V	Q	L	P	G	Y	192
577	TAC	TAC	GTG	GAC	AGC	AAG	CTC	GAC	ATC	ACC	AGC	CAC	AAC	GAG	GAC	TAC	624
193	Y	Y	V	D	S	K	L	D	I	T	S	H	N	E	D	Y	208
TCC (S) for 6/9-2G and 6/9-Q3																	
625	ACC	ATC	GTG	GAG	CAG	TAC	GAG	CGG	ACC	GAG	GGC	CGG	CAC	CAC	CTC	TTC	672
209	T	I	V	E	Q	Y	E	R	<u>T</u>	E	G	R	H	H	L	F	224
673	CTC	TGA															678
225	L	*															226

(SEQ ID NO:11 & 12)

Figure 7

Nucleic acid sequence FP6 (E57)-NA

ATGGCCTCCTCCGAGAACGTCATCACCGAGTTCATGCGCTTCAAGGTGCGCATGGAGGGCACCGTGA
ACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCTACGAGGGCCACAACACCGTG
AAGCTGAAGGTGACCAAGGGCGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCCAGTTCCAGT
ACGGCTCCAAGGTGTACGTGAAGCACCCCGCCGACATCCCCGACTACAAGAAGCTGTCTTCCCCGA
GGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGCGGACCGTGACCCAGGACTC
CTCCCTGCAGGACGGCTGCTTCATCTACAAGGTGAAGTTCATCGGCGTGAAGTTCCTCCGACGGC
CCCGTGATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCACCGAGCGCCTGTACCCCCGCGACGGC
GTGCTGAAGGGCGAGACCCACAAGGCCCTGAAGCTGAAGGACGGCGGCCACTACCTGGTGGAGTTC
AAGTCCATCTACATGGCCAAGAAGCCCGTGAGCTGCCCGGCTACTACTACGTGGACGCCAAGCTGG
ACATCACCTCCCACAACGAGGACTACACCATCGTGGAGCAGTACGAGCGCACCGAGGGCCGCCACCA
CCTGTTCTTG (SEQ ID NO:13)

Figure 8

DNA sequence (ORF) of E5-NA

ATGGCCTCCTCCGAGAACGTCATCACCGAGTTCATGCGCTTCAAGGTGCGCATGGAGGGCACCGTGA
ACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCTACGAGGGCCACAACACCGTG
AAGTTGAAGGTGACCAAGGGCGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCCAGTTCCAGT
ACGGCTCCAAGGTGTACGTGAAGCACCCCGCCGACATCCCCGACTACAAGAAGCTGTCTTCCCCGA
GGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGCGGACCGTGACCCAGGACTC
CTCCCTGCAGGACGGCTGCTTCATCTACAAGGTGAAGTTCATCGGCGTGAAGTTCCTCCGACGGC
CCCGTGATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCACCGAGCGCCTGTACCCCCGCGACGGC
GTGCTGAAGGGCGAGATCCACAAGGCCCTGAAGCTGAAGGACGGCGGCCACTACCTGGTGGAGTTC
AAGTCCATCTACATGGCCAAGAAGCCCGTGAGCTGCCCGGCTACTACTACGTGGACACCAAGCTGG
ACATCACCTCCCACAACGAGGACTACACCATCGTGGAGCAGTACGAGCGCACCGAGGGCCGCCACCA
CCTGTTCTGTAA (SEQ ID NO:14)

Figure 9

Non-aggregating mutant FP3-NA was generated from zFP506-N65M (non-humanized version). In comparison with zFP506-N65M, FP3-NA contains two additional amino acid substitutions - K5E and K10E. Also, one accidental nucleotide substitution was introduced due to PCR mistake (double underline).

Cloning into pQE30 was done using BamHI and HindIII sites:

```

GGA TCC GCT CAG TCA GAG CAC GGT CTA ACA GAA GAA ATG ACA ATG AAA
BamHI  A  Q  S  E  H  G  L  T  E  E  M  T  M  K

TAC CGT ATG GAA GGG TGC GTC GAT GGA CAT AAA TTT GTG ATC ACG GGA
Y  R  M  E  G  C  V  D  G  H  K  F  V  I  T  G

GAG GGC ATT GGA TAT CCG TTC AAA GGG AAA CAG GCT ATT AAT CTG TGT
E  G  I  G  Y  P  F  K  G  K  Q  A  I  N  L  C

GTG GTC GAA GGT GGA CCA TTG CCA TTT GCC GAA GAC ATA TTG TCA GCT
V  V  E  G  G  P  L  P  F  A  E  D  I  L  S  A

GCC TTT ATG TAC GGA AAC AGG GTT TTC ACT GAA TAT CCT CAA GAC ATA
A  F  M  Y  G  N  R  V  F  T  E  Y  P  Q  D  I

GTT GAC TAT TTC AAG AAC TCG TGT CCT GCT GGA TAT ACA TGG GAC AGG
V  D  Y  F  K  N  S  C  P  A  G  Y  T  W  D  R

TCT TTT CTC TTT GAG GAT GGA GCA GTT TGC ATA TGT AAT GCA GAT ATA
S  F  L  F  E  D  G  A  V  C  I  C  N  A  D  I

ACA GTG AGT GTT GAA GAA AAC TGC ATG TAT CAT GAG TCC AAA TTC TAT
T  V  S  V  E  E  N  C  M  Y  H  E  S  K  F  Y

GGA GTG AAT TTT CCT GCT GAT GGA CCT GTG ATG AAA AAG ATG ACA GAT
G  V  N  F  P  A  D  G  P  V  M  K  K  M  T  D

AAC TGG GAG CCA TCC TGC GAG AAG ATC ATA CCA GTA CCT AAG CAG GGG
N  W  E  P  S  C  E  K  I  I  P  V  P  K  Q  G

ATA TTG AAA GGG GAT GTC TCC ATG TAC CTC CTT CTG AAG GAT GGT GGG
I  L  K  G  D  V  S  M  Y  L  L  L  K  D  G  G

CGT TTA CGG TGC CAA TTC GAC ACA GTT TAC AAA GCA AAG TCT GTG CCA
R  L  R  C  Q  F  D  T  V  Y  K  A  K  S  V  P

AGA AAG ATG CCG GAC TGG CAC TTC ATC CAG CAT AAG CTC ACC CGT GAA
R  K  M  P  D  W  H  F  I  Q  H  K  L  T  R  E

GAC CGC AGC GAT GCT AAG AAT CAG AAA TGG CAT CTG ACA GAA CAT GCT
D  R  S  D  A  K  N  Q  K  W  H  L  T  E  H  A

ATT GCA TCC GGA TCT GCA TTG CCC TGA AAGCTT
I  A  S  G  S  A  L  P  *  HindIII  (SEQ ID NO:15 & 16)

```


Figure 10

Non-aggregating mutant FP4-NA was generated from zFP538-M128V (humanized version). In comparison with zFP538-M128V, FP4-NA contains two additional amino acid substitutions - K5E and K9T. Also, two accidental nucleotide substitutions were introduced due to PCR mistakes (double underline).

Cloning into pQE30 was done using BamHI and HindIII sites:

```

GGA TCC GCC CAC AGC GAG CAC GGC CTG ACC GAG GAG ATG ACC ATG AAG
BamHI  A  H  S  E  H  G  L  T  E  E  M  T  M  K

TAC CAC ATG GAG GGC TGC GTG AAC GGC CAC AAG TTC GTG ATC ACC GGC
Y  H  M  E  G  C  V  N  G  H  K  F  V  I  T  G

GAG GGC ATC GGC TAC CCC TTC AAG GGC AAG CAG ACC ATC AAC CTG TGC
E  G  I  G  Y  P  F  K  G  K  Q  T  I  N  L  C

GTG ATC GAG GGC GGC CCC CTG CCC TTC AGC GAG GAC ATC CTG AGC GCC
V  I  E  G  G  P  L  P  F  S  E  D  I  L  S  A

GGC TTC AAG TAC GGC GAC CGG ATC TTC ACC GAG TAC CCC CAG GAC ATC
G  F  K  Y  G  D  R  I  F  T  E  Y  P  Q  D  I

GTG GAC TAC TTC AAG AAC AGC TGC CCC GCC GGC TAC ACC TGG GGC CGG
V  D  Y  F  K  N  S  C  P  A  G  Y  T  W  G  R

AGC TTC CTG TTC GAG GAC GGC GCC GTG TGC ATC TGT AAC GTG GAC ATC
S  F  L  F  E  D  G  A  V  C  I  C  N  V  D  I

ACC GTG AGC GTG AAG GAG AAC TGC ATC TAC CAC AAG AGC ATC TTC AAC
T  V  S  V  K  E  N  C  I  Y  H  K  S  I  F  N

GGC GTG AAC TTC CCC GCC GAC GGC CCC GTG ATG AAG AAG ATG ACC ACC
G  V  N  F  P  A  D  G  P  V  M  K  K  M  T  T

AAC TGG GAG GCC AGC TGC GAG AAG ATC ATG CCC GTG CCT AAG CAG GGC
N  W  E  A  S  C  E  K  I  M  P  V  P  K  Q  G

ATC CTG AAG GGC GAC GTG AGC ATG TAC CTG CTG CTG AAG GAC GGC GGC
I  L  K  G  D  V  S  M  Y  L  L  L  K  D  G  G

CGG TAC CGG TGC CAG TTC GAC ACC GTG TAC AAG GCC AAG AGC GTG CCC
R  Y  R  C  Q  F  D  T  V  Y  K  A  K  S  V  P

AGC AAG ATG CCC GAG TGG CAC TTC ATC CAG CAC AAG CTG CTG CGG GAG
S  K  M  P  E  W  H  F  I  Q  H  K  L  L  R  E

GAC CGG AGC GAC GCC AAG AAC CAG AAG TGG CAG CTG ACC GAG CAC GCC
D  R  S  D  A  K  N  Q  K  W  Q  L  T  E  H  A

ATC GCC TTC CCC AGC GCC CTG GCC TGA AAGCTT
I  A  F  P  S  A  L  A  *  HindIII
  
```

(SEQ ID NOS: 17 & 18)

Figure 11

		M	A	L	S	N	E	F	I	G	D	D	M	K	M
676		ATG	GCC	CTG	TCC	AAC	GAG	TTC	ATC	GGC	GAC	GAC	ATG	AAG	ATG
		TAC	CGG	GAC	AGG	TTG	TTC	AAG	TAG	CCG	CTG	CTG	TAC	TTC	TAC
	T	Y	H	M	D	G	C	V	N	G	H	Y	F	T	V
721	ACC	TAC	CAC	ATG	GAC	GGC	TGC	GTG	AAC	GGC	CAC	TAC	TTC	ACC	GTG
	TGG	ATG	GTG	TAC	CTG	CCG	ACG	CAC	TTG	CCG	GTG	ATG	AAG	TGG	CAC
	K	G	E	G	S	G	K	P	Y	E	G	T	Q	T	S
766	AAG	GGC	GAG	GGC	AGC	GGC	AAG	CCC	TAC	GAG	GGC	ACC	CAG	ACC	TCC
	TTC	CCG	CTC	CCG	TCG	CCG	TTC	GGG	ATG	CTC	CCG	TGG	GTC	TGG	AGG
	T	F	K	V	T	M	A	N	G	G	P	L	A	F	S
811	ACC	TTC	AAG	GTG	ACC	ATG	GCC	AAC	GGC	GGC	CCC	CTG	GCC	TTC	TCC
	TGG	AAG	TTC	CAC	TGG	TAC	CGG	TTG	CCG	CCG	GGG	GAC	CGG	AAG	AGG
	F	D	I	L	S	T	V	F	M	Y	G	N	R	C	F
856	TTC	GAC	ATC	CTG	TCC	ACC	GTG	TTC	ATG	TAC	GGC	AAC	CGC	TGC	TTC
	AAG	CTG	TAG	GAC	AGG	TGG	CAC	AAG	TAC	ATG	CCG	TTG	GCG	ACG	AAG
	T	A	Y	P	T	S	M	P	D	Y	F	K	Q	A	F
901	ACC	GCC	TAC	CCC	ACC	AGC	ATG	CCC	GAC	TAC	TTC	AAG	CAG	GCC	TTC
	TGG	CGG	ATG	GGG	TGG	TCG	TAC	GGG	CTG	ATG	AAG	TTC	GTC	CGG	AAG
	P	D	G	M	S	Y	E	R	T	F	T	Y	E	D	G
946	CCC	GAC	GGC	ATG	TCC	TAC	GAG	AGA	ACC	TTC	ACC	TAC	GAG	GAC	GGC
	GGG	CTG	CCG	TAC	AGG	ATG	CTC	TCT	TGG	AAG	TGG	ATG	CTC	CTG	CCG
	G	V	A	T	A	S	W	E	I	S	L	K	G	N	C
991	GGC	GTG	GCC	ACC	GCC	AGC	TGG	GAG	ATC	AGC	CTG	AAG	GGC	AAC	TGC
	CCG	CAC	CGG	TGG	CGG	TCG	ACC	CTC	TAG	TCG	GAC	TTC	CCG	TTG	ACG
	F	E	H	K	S	T	F	H	G	V	N	F	P	A	D
1036	TTC	GAG	CAC	AAG	TCC	ACC	TTC	CAC	GGC	GTG	AAC	TTC	CCC	GCC	GAC
	AAG	CTC	GTG	TTC	AGG	TGG	AAG	GTG	CCG	CAC	TTG	AAG	GGG	CGG	CTG
	G	P	V	M	A	K	K	T	T	G	W	D	P	S	F
1081	GGC	CCC	GTG	ATG	GCC	AAG	AAG	ACC	ACC	GGC	TGG	GAC	CCC	TCC	TTC
	CCG	GGG	CAC	TAC	CGG	TTC	TTC	TGG	TGG	CCG	ACC	CTG	GGG	AGG	AAG
	E	K	M	T	V	C	D	G	I	L	K	G	D	V	T
1126	GAG	AAG	ATG	ACC	GTG	TGC	GAC	GGC	ATC	TTG	AAG	GGC	GAC	GTG	ACC
	CTC	TTC	TAC	TGG	CAC	ACG	CTG	CCG	TAG	AAC	TTC	CCG	CTG	CAC	TGG
	A	F	L	M	L	Q	G	G	G	N	Y	R	C	Q	F
1171	GCC	TTC	CTG	ATG	CTG	CAG	GGC	GGC	GGC	AAC	TAC	AGA	TGC	CAG	TTC
	CGG	AAG	GAC	TAC	GAC	GTC	CCG	CCG	CCG	TTG	ATG	TCT	ACG	GTC	AAG
	H	T	S	Y	K	T	K	K	P	V	T	M	P	P	N
1216	CAC	ACC	TCC	TAC	AAG	ACC	AAG	AAG	CCC	GTG	ACC	ATG	CCC	CCC	AAC
	GTG	TGG	AGG	ATG	TTC	TGG	TTC	TTC	GGG	CAC	TGG	TAC	GGG	GGG	TTG
	H	V	V	E	H	R	I	A	R	T	D	L	D	K	G
1261	CAC	GTG	GTG	GAG	CAC	CGC	ATC	GCC	AGA	ACC	GAC	CTG	GAC	AAG	GGC
	GTG	CAC	CAC	CTC	GTG	GCG	TAG	CGG	TCT	TGG	CTG	GAC	CTG	TTC	CCG
	G	N	S	V	Q	L	T	E	H	A	V	A	H	I	T
1306	GGC	AAC	AGC	GTG	CAG	CTG	ACC	GAG	CAC	GCC	GTG	GCC	CAC	ATC	ACC
	CCG	TTG	TCG	CAC	GTC	GAC	TGG	CTC	GTG	CGG	CAC	CGG	GTG	TAG	TGG
	S	V	V	P	F	*									
1351	TCC	GTG	GTG	CCC	TTC	TGA									
	AGG	CAC	CAC	GGG	AAG	ACT									

(SEQ ID NO:19 & 20)

Figure 12

Non-aggregating mutant FP7-NA was generated from M35-5 (FP7a). In comparison with M35-5, FP7-NA contains two additional substitutions - K6T and K7E. Nucleotide substitutions in the codon for Leu-4 were introduced to optimize codon usage (double underline).

Cloning into pQE30 was done using BamHI and HindIII sites:

<u>GGA</u>	<u>TCC</u>	GCC	TCC	<u>CTG</u>	CTG	ACC	GAG	ACC	ATG	CCC	TTC	AGG	ACC	ACC	ATC
BamHI		A	S	<u>L</u>	L	T	E	T	M	P	F	R	T	T	I
GAG	GGC	ACC	GTG	AAC	GGC	CAC	TAC	TTC	AAG	TGC	ACC	GGC	AAG	GGC	GAG
E	G	T	V	N	G	H	Y	F	K	C	T	G	K	G	E
GGC	AAC	CCC	CTC	GAG	GGC	ACC	CAG	GAG	ATG	AAG	ATC	GAG	GTG	ATC	GAG
G	N	P	L	E	G	T	Q	E	M	K	I	E	V	I	E
GGC	GGC	CCC	CTG	CCC	TTC	GCC	TTC	CAC	ATC	CTG	TCC	ACC	TCC	TGC	ATG
G	G	P	L	P	F	A	F	H	I	L	S	T	S	C	M
TAC	GGC	TCC	AAG	GCC	TTC	ATC	AAG	TAC	GTG	TCC	GGC	ATC	CCC	GAC	TAC
Y	G	S	K	A	F	I	K	Y	V	S	G	I	P	D	Y
TTC	AAG	CAG	TCC	CTC	CCC	GAG	GGC	TTC	ACC	TGG	GAG	CGC	ACC	ACC	ACC
F	K	Q	S	L	P	E	G	F	T	W	E	R	T	T	T
TAC	GAG	GAC	GGC	GGC	TTC	CTG	ACC	GCC	CAC	CAG	GAC	ACC	TCC	CTG	GAC
Y	E	D	G	G	F	L	T	A	H	Q	D	T	S	L	D
GGC	GAC	TGC	CTG	GTG	TAC	AAG	GTG	AAG	ATC	CTG	GGC	AAC	AAC	TTC	CCC
G	D	C	L	V	Y	K	V	K	I	L	G	N	N	F	P
GCC	GAC	GGC	CCC	GTG	ATG	CAG	AAC	AAG	GCC	GGC	CGC	TGG	GAG	CCC	TCC
A	D	G	P	V	M	Q	N	K	A	G	R	W	E	P	S
ACC	GAG	ATC	GTG	TAC	GAG	GTG	GAC	GGC	GTG	CTG	CGC	GGC	CAG	TCC	CTG
T	E	I	V	Y	E	V	D	G	V	L	R	G	Q	S	L
ATG	GCC	CTG	GAG	TGC	CCC	GGC	GGT	CGC	CAC	CTG	ACC	TGC	CAC	CTG	CAC
M	A	L	E	C	P	G	G	R	H	L	T	C	H	L	H
ACC	ACC	TAC	CGC	TCC	AAG	AAG	CCC	GCC	TCC	GCC	CTG	AAG	ATG	CCC	GGC
T	T	Y	R	S	K	K	P	A	S	A	L	K	M	P	G
TTC	CAC	TTC	GAG	GAC	CAC	CGC	ATC	GAG	ATC	CTG	GAG	GAG	GTG	GAG	AAG
F	H	F	E	D	H	R	I	E	I	L	E	E	V	E	K
GGC	AAG	TGC	TAC	AAG	CAG	TAC	GAG	GCC	GCC	GTG	GGC	CGC	TAC	TGC	GAC
G	K	C	Y	K	Q	Y	E	A	A	V	G	R	Y	C	D
GCC	GCC	CCC	TCC	AAG	CTG	GGC	CAC	AAC	<u>TGAAGCTT</u>						
A	A	P	S	K	L	G	H	N	*	HindIII (SEQ ID NO:21 & 22)					

Figure 13

Dimeric mutant AsRed M35-5D (in comparison to M355NA it carries one amino acid substitution L166S according to GFP numbering; L159S according to self-numbering)

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      ATG GCC TCC CTG CTG ACC GAG ACC ATG CCC TTC AGG ACC ACC ATC
      M  A  S  L  L  T  E  T  M  P  F  R  T  T  I

GAG GGC ACC GTG AAC GGC CAC TAC TTC AAG TGC ACC GGC AAG GGC GAG
E  G  T  V  N  G  H  Y  F  K  C  T  G  K  G  E

GGC AAC CCC CTC GAG GGC ACC CAG GAG ATG AAG ATC GAG GTG ATC GAG
G  N  P  L  E  G  T  Q  E  M  K  I  E  V  I  E

GGC GGC CCC CTG CCC TTC GCC TTC CAC ATC CTG TCC ACC TCC TGC ATG
G  G  P  L  P  F  A  F  H  I  L  S  T  S  C  M

TAC GGC TCC AAG GCC TTC ATC AAG TAC GTG TCC GGC ATC CCC GAC TAC
Y  G  S  K  A  F  I  K  Y  V  S  G  I  P  D  Y

TTC AAG CAG TCC CTC CCC GAG GGC TTC ACC TGG GAG CGC ACC ACC ACC
F  K  Q  S  L  P  E  G  F  T  W  E  R  T  T  T

TAC GAG GAC GGC GGC TTC CTG ACC GCC CAC CAG GAC ACC TCC CTG GAC
Y  E  D  G  G  F  L  T  A  H  Q  D  T  S  L  D

GGC GAC TGC CTG GTG TAC AAG GTG AAG ATC CTG GGC AAC AAC TTC CCC
G  D  C  L  V  Y  K  V  K  I  L  G  N  N  F  P

GCC GAC GGC CCC GTG ATG CAG AAC AAG GCC GGC CGC TGG GAG CCC TCC
A  D  G  P  V  M  Q  N  K  A  G  R  W  E  P  S

ACC GAG ATC GTG TAC GAG GTG GAC GGC GTG CTG CGC GGC CAG TCC AGC
T  E  I  V  Y  E  V  D  G  V  L  R  G  Q  S  S

ATG GCC CTG GAG TGC CCC GGC GGT CGC CAC CTG ACC TGC CAC CTG CAC
M  A  L  E  C  P  G  G  R  H  L  T  C  H  L  H

ACC ACC TAC CGC TCC AAG AAG CCC GCC TCC GCC CTG AAG ATG CCC GGC
T  T  Y  R  S  K  K  P  A  S  A  L  K  M  P  G

TTC CAC TTC GAG GAC CAC CGC ATC GAG ATC CTG GAG GAG GTG GAG AAG
F  H  F  E  D  H  R  I  E  I  L  E  E  V  E  K

GGC AAG TGC TAC AAG CAG TAC GAG GCC GCC GTG GGC CGC TAC TGC GAC
G  K  C  Y  K  Q  Y  E  A  A  V  G  R  Y  C  D

GCC GCC CCC TCC AAG CTG GGC CAC AAC TGA
A  A  P  S  K  L  G  H  N  *

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(SEQ ID NO:23 & 24)

Figure 15

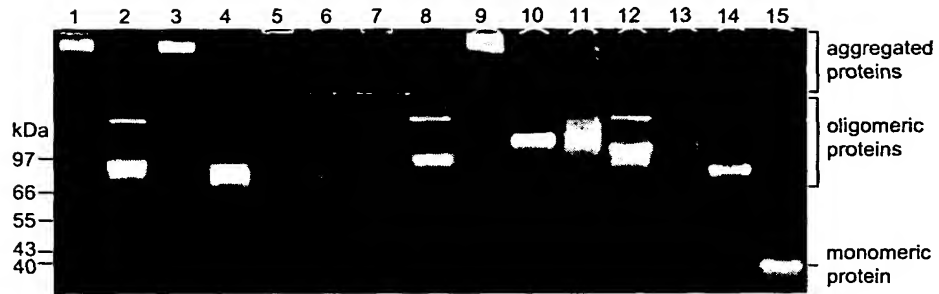


Figure 16

